

Claims

1. A method for purifying recombinantly produced angiostatin comprising:

5 applying crude fermentation broth containing the recombinantly produced angiostatin to an expanded bed cation exchange column;

collecting eluate from the expanded bed cation exchange column and applying the eluate from the expanded bed cation exchange column to an anion exchange column;

10 collecting eluate from the anion exchange column and applying the eluate from the anion exchange column to a hydroxyapatite column;

collecting eluate from the hydroxyapatite column and applying the eluate from the hydroxyapatite column to a hydrophobic column;

15 collecting eluate from the hydrophobic column and applying the eluate from the hydrophobic column to a membrane; and collecting fluid passing through the membrane.

2. The method of Claim 1 further comprising:

concentrating the fluid;

diafiltering the fluid;

concentrating the diafiltered fluid; and

20 passing the concentrated and diafiltered fluid through a second membrane.

3. The method of Claim 2, further comprising:

collecting fluid passing through the second membrane;

concentrating the fluid passing through the second membrane; and

25 aseptically filtering the concentrated fluid.

4. The method of Claim 1, wherein the recombinantly produced angiostatin is human angiostatin.

5. The method of Claim 4, wherein the recombinantly produced human angiostatin is produced from fermentation of *Pichia pastoris*.

6. The method of Claim 1, wherein the crude fermentation broth containing the recombinantly produced angiostatin is applied to the expanded bed cation exchange column in an upward direction, followed by application of elution buffer and elution of the column in a downward direction.

5

TOP SECRET